Scientific Abstract

Strategies to stimulate T cell response for cancer treatment have been explored intensively using melanoma as model system. Clinical trials employing dendritic cell (DC)- based tumor vaccines have demonstrated their ability to induce cytotoxic T lymphocyte (CTL) response and tumor nodule regression in some patients. Although those trials proved the principle of such approach to treating melanoma, most nodules continued to progress. This may be due to two major reasons: the heterogeneity of the tumor cells and an insufficient level of CTL generation. The problem of heterogeneity may be circumvented by immunization against multiple melanoma antigens to cover different subsets of melanoma cells, and the CTL level can be boosted by recruiting CD4 cells for the stimulation.

We designed a strategy to address those problems. In our protocol, DCs will be transfected with multiple species of in vitro-transcribed mRNA encoding melanoma tumor antigens and will be engineered to have LAMP signals to facilitate a class II pathway antigen presentation (RNA-DC). We have conducted preclinical studies in HLA-A2 volunteers and verified that mature DCs transfected with multiple species of melanoma antigens are capable of presenting corresponding epitopes to CTLs. In addition, the LAMP sequence has been shown to be able to present the antigens to the Class II pathway in mature DCs. We have also shown in vitro the superiority of the transfected DCs to the most commonly used peptide-pulsed DCs in terms of both level of CTLs and T cell receptor (TCR) avidity. The hypothesis to be tested in this protocol is that administration of such DCs is safe and can stimulate a high level of and a high TCR avidity of the CTL response to multiple melanoma tumor antigens.

The primary objectives of this protocol are: 1) to determine the maximal tolerable dose (MTD) of the RNA-DC vaccine in patients with Stages III and IV melanoma, and 2) to determine optimal biologic dose of the RNA-DC vaccine capable of stimulating CD4 and CD8 T cell responses against multiple antigens. Patients with appropriate HLA alleles will be evaluated for expression of Mart-1, Tyrosinase, gp100 and MAGE-3 antigens in their tumors. We plan to enroll 12 to 30 patients and to immunize them with autologous mature DCs transfected with a mixture of RNAs encoding the antigens intra-nodally at four different dose levels at 2 weeks intervals for a total of four doses. We propose to monitor the safety and toxicity of the vaccine for determination of MTD. We propose to collect blood before and after treatment to determine the responses of the CTLs to the immunized melanoma tumor antigens by using the ELISPOT technique. If necessary, we propose to carry out a dose escalation to achieve CTL responses against maximal number of antigens and epitopes. For patients with the HLA-A2 allele, we propose to analyze TCR avidity with HLA-A2-restricted tetramers. In addition, in patients who have a high level of CTL response and yet have progressing tumor nodules, we propose to obtain biopsies to evaluate the sensitivity of such nodules to the stimulated CTLs using a newly established technique. This proposed project will establish a platform for studying the critically important relationship between stimulated CTLs and autologous tumor targets. Our long-term goal is to further explore this platform to study issues such as CTL expansion control by various factors in vivo, avoidance of T cell anergy, and antigen modification in target tumor cells.